Promoting Factors of Turkey Meat Products Oxidation

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Abstract

With the purpose of deepening the knowledge on promoting factors of meat discoloration, an experiment was conducted in a Portuguese meat industry with a specific product, turkey skewers (TS), where the discoloration was recurrent. The causes behind the oxidation of meat products were addressed, focusing on those of industrial origin, evidencing primarily the stages of processing, packaging and storage of final product. The experiment consisted on three assays and each one focused a potential oxidation promoting factor within a stage of the technological process. The first assay (F) was conducted to compare two different packaging materials with different oxygen (O_2) transmission rate values: F1 – 53.9 $cm^3/m^2/24h$ and F2 – 5 $cm^3/m^2/24h$. The second assay (C) was performed to study the influence of packaging atmosphere/meat *ratio* on colour: C1 with a *ratio* of 1.16 and C2 of 2.11. The third assay (L) focused on the influence of two light conditions with L1 being the standard light storage (25 Watt LED light bulb) and L2 the storage in the dark. To assess product alterations, parameters – oxygen (O_2), carbon dioxide (CO_2), pH, a_W, colour (L*, a* and b* values), oxidation stability – thiobarbituric acid reactive substances (TBARS value), coliforms, total mesophilic aerobic microorganisms, lactic acid bacteria, moulds, yeasts, *Salmonella* spp., *Listeria monocytogenes, Escherichia coli* and sensory evaluation – were assessed along shelf-life period (7 days, 3 ± 2 ºC).

Assay 1 showed the most relevant results, reflecting the influence of packaging materials on meat colour. F2 revealed abnormal packaging atmosphere composition measurements in comparison to F1. The packages differed significantly from each other in relation to pH (*p*-value <0.05) and displayed relevant differences on sensory evaluation, colour and TBARS. It was concluded a more rapid degradation of F2. The results obtained in the others assays did not allowed such precise conclusions to be drawn and therefore the packaging material was considered to be the factor with greatest impact on meat products oxidation.

Keywords: discoloration, packaging material, light, meat-atmosphere proportion, turkey meat

Resumo

Com o objectivo de aprofundar o tema factores promotores da oxidação da carne, foi realizada uma experiência numa unidade de processamento de carne, focando espetadas de peru. As causas da oxidação da carne foram consideradas, com especial enfoque para as de origem industrial, evidenciando as fases de processamento, embalamento e armazenamento. A experiência consistiu em três ensaios, cada um focado num factor promotor da oxidação da carne. O primeiro ensaio (F) baseou-se no tipo de embalagem, comparando dois tipos de filme, com diferentes taxas de transmissão de oxigénio: F1 – 53.9 $cm^3/m^2/24h$ e F2 – 5 $cm^3/m^2/24h$. O segundo ensaio (C) baseou-se na proporção atmosfera/carne, em que C1 corresponde a uma cuvete *standard,* com proporção 1.16 e C2 corresponde a uma cuvete alternativa, com proporção 2.11. No terceiro ensaio (L) baseou-se no armazenamento de produto acabado sob dois tipos de iluminação, L1 – luz *standard* (25 *Watt LED*) e L2 – sem luz. Certos parâmetros foram utilizados para medir alterações: O_2, CO_2, pH, a_w e avaliação sensorial; cor – através do sistema *CIE Lab* que mede os parâmetros L*, a* e b*, e TBARS (*thiobarbituric acid reactive substances*); análises microbiológicas: pesquisa e contagem de Coliformes (Col), Mesófilos Aeróbios Totais (Mots), contagem de Bactérias Ácido Lácticas (Lac), contagem de Leveduras (Lev) e Bolores e pesquisa de *E. coli, Salmonella spp e L. monocytogenes*).

O ensaio 1 mostrou maior relevância, reflectindo a influência dos materiais de embalagem na cor da carne. F2 revelou comportamento anormal da composição atmosférica, comparando com F1. As embalagens diferiram significativamente em relação ao pH (*p*-value <0.05) e mostraram alterações acentuadas a nível visual, análise da cor e *TBARS*. Concluiu-se maior degradação de F2. Os resultados dos outros ensaios não permitiram alcançar conclusões tão concretas e acentuadas, concluindo-se deste modo que o material de embalagem foi o factor com impacto mais significativo.

Palavras-chave: alteração de cor, material de embalagem, luz, proporção atmosfera/carne, carne de peru

Resumo Alargado

Todos os alimentos frescos têm, no geral, um prazo de validade curto. A carne não é excepção, sendo o prazo de aproximadamente uma semana, para a maioria da carne fresca refrigerada e embalada em atmosfera protectora. Dos principais atributos de qualidade que caracterizam os alimentos, a cor é um dos mais importantes e é aquele que no momento da compra mais influencia a decisão do consumidor. Ao comprar um produto embalado fresco não se consegue avaliar características como o odor e/ou o sabor do alimento, e é por isso que estes produtos são embalados de modo a poderem ser visíveis, tendo a cor, e consequentemente a aparência, um papel relevante no momento da compra. Assim, é de extrema importância preservar as características da carne, o seu aspecto e mais especificamente a cor, sendo desejável a cor "vermelho vivo" associada a uma carne mais fresca (tendo esta associação maior relevância para carnes vermelhas). A cor da carne deve-se em parte à concentração em mioglobina (Mb), a proteína da carne responsável pela cor avermelhada (Sgarbieri, 1996). Ora acontece que a Mb é vermelha no seu estado natural, mas, devido a alterações provocadas por agentes externos como a luz, oxigénio e elevadas temperaturas, rapidamente reage dando origem às formas oxidadas como é o caso da metamioglobina. Este processo oxidativo da Mb é caracterizado pela mudança de cor, de vermelho vivo para cores mais escuras como roxo, castanho e cinzento (Fletcher, 1999). A degradação oxidativa dos alimentos frescos tem sido uma problemática para a indústria alimentar no geral e, mais especificamente, no sector das carnes, uma vez que a alteração de cor da carne constitui um grave problema com consequências económicas, comerciais e a nível de imagem da marca para as empresas.

Com o objectivo de saber mais sobre a temática da oxidação da carne e o seu efeito nas alterações da cor desta, foi realizado um estudo sobre os factores promotores da oxidação de produtos à base de carne de peru, numa grande indústria de processamento de carne de aves a operar em Portugal. O estudo incidiu sobre espetadas de peru embaladas numa cuvete de poliestireno expandido termoselada com filme transparente ou timbrado e sob atmosfera protectora (oxigénio, 70% e dióxido de carbono, 30%). Cada cuvete contém quatro espetadas, sendo estas compostas por cubos de carne fresca da coxa de peru intercalados com tiras de pimento verde e toucinho.

O estudo consistiu na realização de três ensaios:

No primeiro ensaio estudou-se a influência do material de embalagem utilizado no embalamento, filme transparente e filme timbrado (F1 – filme liso, F2 – filme timbrado),

sendo a taxa de transmissão de oxigénio (OTR) a característica com valores mais distintos entre os dois filmes (F1 – 53.9 $cm^3/m^2/24h$ e F2 – 5 $cm^3/m^2/24h$).

No segundo ensaio comparou-se os efeitos de duas proporções atmosfera/carne (C1 – 1.16; C2 – 2.11), em que C1 corresponde a uma cuvete *standard* com quatro espetadas e C2 corresponde a uma cuvete alternativa com três espetadas.

No terceiro ensaio analisou-se o comportamento do produto final armazenado sob dois tipos de iluminação (L1 – com luz, L2 – sem luz) sendo que L1 correspondeu a cuvetes armazenadas simulando o expositor do supermercado e L2 a cuvetes armazenadas em caixa opaca à luz simulando armazenagem na ausência de luz.

Nos três ensaios procedeu-se à medição de vários parâmetros ao longo do tempo de vida útil do produto (7 dias, 3 ± 2ºC). Os parâmetros estudados foram: a composição atmosférica da embalagem (O_2 e CO_2), pH, a_w e avaliação sensorial (aspecto visual) (estes parâmetros foram medidos todos os dias durante o prazo de validade estabelecido); cor – através do sistema *CIE Lab* que mede os parâmetros L*, a* e b*, e TBARS (*thiobarbituric acid reactive substances*) – medidos no primeiro e último dia do prazo de validade (D0 e D7); análises microbiológicas – efectuadas nos dias 0,3,5 e 7 (D0, D3, D5 e D7) (pesquisa e contagem de Coliformes (Col), Mesófilos Aeróbios Totais (Mots), contagem de Bactérias Ácido Lácticas (Lac), contagem de Leveduras (Lev) e Bolores e pesquisa de *E. coli, Salmonella spp e L. monocytogenes*).
Após realização dos ensaios, procedeu-se à análise e tratamento dos resultados, sendo que os parâmetros foram todos avaliados recorrendo à organização dos resultados em gráficos e tabelas e os parâmetros O_2, CO_2, pH e a_W foram posteriormente tratados estatisticamente recorrendo ao software de tratamento estatístico R.

Os parâmetros em que se notou maior discrepância entre amostras foram: gases da embalagem, oxigénio (O_2) e dióxido de carbono (CO_2), parâmetros da cor a* e b*, indicador da estabilidade oxidativa, TBARS e avaliação sensorial; enquanto que para os parâmetros a_W, parâmetro da cor L* e contagens microbiológicas as diferenças entre os resultados das amostras em comparação foram menos relevantes, sendo que para o a_W obteve-se muitas vezes valores muito semelhantes para as amostras das diferentes embalagens do mesmo ensaio e, houve mesmo amostras com valores iguais, como no caso das medições do dia 5 em que os valores para o ensaio 2, relativo à proporção atmosfera/carne são iguais entre si e muito semelhantes aos obtidos para o ensaio 3, relativo ao tipo de luz no armazenamento (a_{WF1}=0.771 , a_{WF2}=0.777; a_{WC1}=0.647, a_{WC2}=0.647; a_{WL1}=0.672, a_{WL2}=0.671).

No primeiro ensaio, verificaram-se diferenças entre as amostras em estudo principalmente nos parâmetros composição atmosférica, TBARS e avaliação sensorial. A amostra com o filme timbrado (F2) registou na medição dos gases da embalagem uma variação irregular para o valor de CO_2 ao longo do período analisado; as amostras apresentaram variação de pH significativamente diferente uma da outra (*p*-value <0.05). Ambas as amostras apresentaram valores elevados no que concerne às análises microbiológicas, alcançando para os Mesófilos Aeróbios Totais (Mots) valores superiores a 7 log cfu/g nos dias 5 e 7; relativamente aos parâmetros da cor, TBARS e avaliação sensorial as diferenças são evidentes, sendo que F2 apresenta alteração de cor e odor visíveis logo no dia 4 e formação de subprodutos de oxidação lipídica mais elevada ($F2_{TBARS}$=1.533 em comparação a $F1_{TBARS}$=1.137), traduzindo-se na degradação mais acentuada e rápida da amostra F2 ao nível do odor, da cor (aparência) e da textura. Para os restantes ensaios não se verificaram diferenças tão marcantes entre amostras, querendo isto dizer que as variantes – proporção de atmosfera/carne e tipo de iluminação na armazenagem – não influenciaram consideravelmente o produto em questão. Ainda assim, foi possível concluir para o ensaio 2, relativo à proporção atmosfera/carne, que a embalagem com proporção 2,11 (C2) apresentou uma cor mais vermelha, através da medição do parâmetro a*, verificando-se um ligeiro aumento no fim do período em estudo (7 dias) e, para a mesma embalagem, também se confirmou maior estabilidade oxidativa pois apresentou menor valor no teste de TBARS, em oposição à embalagem com proporção 1.16 ($C1_{TBARS}$=1.339; $C2_{TBARS}$=0.468). Através da observação do gráfico do dióxido de carbono parece não ter havido diferença entre as embalagens, porém, através do tratamento estatístico verificou-se que as medições de C1 e C2 eram significativamente diferentes (*p*-value <0.05). Relativamente ao ensaio 3, observou-se para a embalagem armazenada sem luz (L2) um aumento acentuado do valor de CO_2 do dia 2 para o dia 3. Na medição do parâmetro b*, verificou-se uma diminuição para L1 (embalagem armazenada com luz) e um aumento para L2 ($L0_{b^*}$=6.13; $L1_{b^*}$=5.35; $L2_{b^*}$=6.19). Também se constatou, para L2, um aumento mais pronunciado dos TBARS ($L1_{TBARS}$= 0.657; $L2_{TBARS}$= 1.158) levando a crer que a ausência de luz promove a oxidação da carne de peru e uma ligeira tendência para a alteração da cor (mais amarelado).

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Abbreviations and Acronyms

a_w – Water Activity
CCP –Critical Control Point
CFU – Colony Forming Units
COMb – Carboxymyoglobin
DeoxyMb – Deoxymyoglobin
DFD – Dark, Firm and Dry
EPS – Expanded Polystyrene
FEFO – First Expire-First Out
FIFO – First In-First Out
FP – Final Product
FSA – Food Standards Agency
GMP – Good Manufacturing Practices
GHP – Good Hygiene Practices
HACCP – Hazard Analysis and Critical Control Point
ISO – International Organization for Standardization
LED – Light Emitting Diode
MDA – Malondialdehyde
MAP – Modified Atmosphere Packaging
Mb – Myoglobin
MetMb – Metmyoglobin
NP – Norma Portuguesa (Portuguese regulation)
OxyMb – Oxymyoglobin
PCA – Plate Count Agar
PSE – Pale, Soft and Exudative
RM – Raw Materials
SMD – Surface Mounted Device
TBA – Thiobarbituric acid
TBARS – Thiobarbituric Acid Reactive Substances
TBX – Tryptone Bile X-Glucuronide
TCA – Trichloroacetic acid
TS – Turkey Skewers
WV – Water Vapour

1. Introduction

Poultry products are universally popular and animals like chicken, quail and turkey, the three most important poultry birds, are domesticated by humans for their eggs, meat or even their feathers. These animals are of high value to the food industry due to their cheap feed conversion rate (FCR) and also by their healthy nutritional composition, high in protein and low in fats, which can be proven by annex 1. In recent years the consumption of poultry meat has risen drastically – two main reasons for this are the lower prices of poultry meat, compared to red meats and the bovine spongiform encephalopathy (BSE) scandal affecting consumption of red meats. To ensure the continued growth and competitiveness of this industry, it is essential that poultry meat quality and safety are maintained during production and processing (Mead, 2004). Turkey meat, in particular, is known for being one of the healthiest meats, containing less calories and fat than most other types of meat compared to chicken, beef and pork (table 1). Poultry is consumed on a worldwide basis, in which differences in ethnic, religious and local customs have resulted in a multitude of ways that meat and other edible parts can be prepared for consumption (Mead, 2004). Turkey is very versatile when it comes to cooking: it can be prepared in various ways such as roasted, fried, grilled, stuffed, among others. One of these ways is like turkey skewers (TS), adding vegetables between the meat, like tomato, onion, green pepper, pineapple or bacon and chorizo. TS are usually grilled; this way of cooking is seen all over the world, with slight differences in the ingredients used or in the way which are cooked.

TS, as a further processing poultry product, have been marketed on large surfaces more recently, as a way to respond to consumers' demands and are packed in modified atmosphere in order to preserve their own nutritional and physic-chemical characteristics delaying deterioration (Mead, 2004). Fresh meat preservation is a problem that concerns all meat industries because oxidation is a natural process of food degradation and, at the present, it is only possible to slow it. The ultimate aim of the industry is to delay the deterioration of meat as much as possible, with the lowest cost, achieving a higher quality for a longer period of time, thus avoiding loss of commercial value and food waste. However, there are ways of preserving the physicochemical characteristics of the meat for a longer time, with the use of modified atmosphere, low temperatures and suitable packaging materials. It is in the best interest of the industry to preserve the characteristics of the meat as long as possible with the means available, in the most efficient and economical way.

The main objective of this work was to study the promoting factors of turkey meat products oxidation. With this purpose, three assays were carried out, to determine the influence of the packaging material, atmosphere/meat *ratio* and storage light.

The three assays included the analysis of several parameters taking into account the following: packaging atmosphere, and more specifically O_2 and CO_2, pH, aW, TBARS, colour and microbiology.

Table 1 – Nutritional values for turkey, chicken, beef and pork (INSA, 2017).

Meat Type	Energy (kcal) [2]	Fat (g) [2]	Protein (g) [2]
Turkey[1]	137	6.1	20.5
Chicken[1]	201	13.6	19.6
Beef[1]	122	4.3	20.9
Pork[1]	190	12.4	19.6

[1] Data based on the nutritional values for whole turkey raw with skin, whole chicken raw with skin, beef steak average value of sparerib, rump and loin) raw and raw pork rib.
[2] Values per 100g edible portion.

1.1 History and Characterization of Turkey

Turkey domestication occurred since 16th century when the animals were first taken from Mexico to Spain and then from there to England, where it gained the name “turkey”, which was formerly used for the guinea fowl of Islamic (or “Turkish”) lands. English colonists then took the animal to North America in the 17th century where it was mainly bred for its feathers, after which the breeding emphasis changed to its meat qualities. Turkey is much appreciated, being an important tradition in many European countries at Christmas and at Thanksgiving holiday in the United States.

The male turkey has 130 cm long and a weigh of about 10 kg. Domesticated strains of the common turkey, developed at industrial level for slaughter purposes, could be heavier. The most important turkey meat cuts are breast, wing and leg. Most common turkey cuts like breast, thigh, drumstick, wing, are presented in figure 1. For the turkey skewers (TS) production is used the leg cut. It subdivides in thigh and drumstick and the focus is on the thigh muscle.

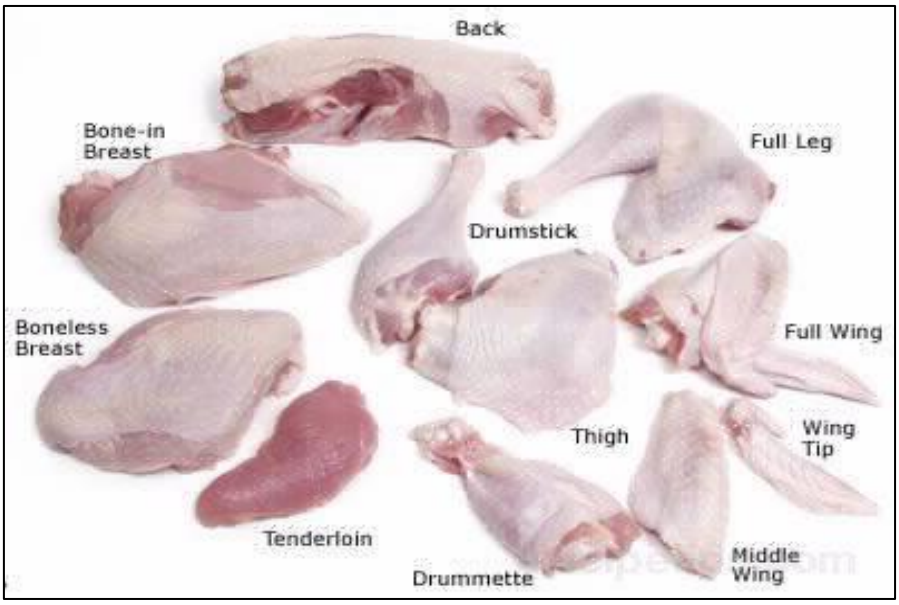

Figure 1 – Turkey meat cuts (Waltkoch, 2018).

1.2 Characterization and Quality of poultry meat products

In Portugal, according to Regulation (EC) nº 853/2004, fresh meat is defined as "meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-packaged or packaged in modified atmosphere". Turkey skewers comply, in part, with the previous statement, but it is also meat that was processed at some point and have other food products (lard and green peppers cuts) added. Hence, from a technical and legal point of view, TS are, by definition from the same regulation, a meat preparation, which is defined as "fresh meat, including meat that has been reduced to fragments, which was added seasonings or additives or which has undergone processes insufficient to modify the internal muscle fiber structure of the meat and thus to eliminate the characteristics of fresh meat". Bearing the definitions, in the course of writing turkey skewers will be referred as "meat" and/or "fresh meat", regardless of the legal definition of "meat preparation".

Poultry meat quality is affected by weather, genotype, rearing conditions, production techniques, transportation, animals' ability to respond to environmental conditions (flooring, temperature, light, flocking density) and all the variables that may interact, influencing the production cycle (Bertol, 2004). The processes preceding industrial technological operations, like breeding factors (age, genotype, sex, rearing conditions) and farming practices, have shown a relevant impact on meat products quality. Several studies concluded that these factors affect the chemical composition of muscle, its structure and metabolism and,

therefore, the mechanisms involved in turning muscle to meat. Singh & Essary (1974) stated that increasing the slaughter age for broilers increases the protein content of breast and thigh meat; also, a decrease in tenderness with advancing age has been reported for turkey breast meat (Ngoka et al., 1982), and Mead (2004), affirmed that the sensory quality of meat is closely related to animal age at slaughter. Sosnicki et al. (1998) reported that, for turkey, there is some evidence that paleness of breast meat could be a consequence of the combination of accelerated *rigor mortis* and high muscle temperature after slaughter, which typically causes protein denaturation leading to pale, soft, exudative (PSE) meat with poorer functionality. Such operations, which were not considered in the present study since it has its focus on industry operations, should not be overlooked and should be considered in order to understand its influence on meat quality and more precisely as oxidation promoting factors.

1.3 Colour of poultry meat

Of the several quality attributes of fresh meat, colour is the most important one, influencing purchase decisions (Mancini & Hunt, 2005).

Meat colour is a thoroughly studied subject. The major contributing factors to poultry meat colour are myoglobin (Mb) content, its chemical state and degradation reactions and meat pH. The main difference in coloration between meats lies essentially in the concentration of Mb, a protein found in the sarcoplasm of skeletal muscle fibers (Sgarbieri, 1996). Wideman *et al.* (2016) referred that the darker colour of leg and more precisely thigh meat is due to the larger amount of Mb and haem pigments, as well as a higher pH when compared to breast meat. Mb content is primarily related to species, muscle and age of the animal (Coulate, 1984) (table 2). Froning et al. (1978) concluded that, on turkeys, Mb concentration varies with the muscle, with concentrations increasing on leg muscle ($breast_{Mb\ concentration}$=0.50 mg/g; $thigh_{Mb\ concentration}$=2.00 mg/g) (table 2).

Muscle pH, which had been shown to be essentially related to the biochemical state of the muscle at time of slaughter and following *rigor mortis* development, affects both the light reflectance properties of the meat as well as the chemical reactions of Mb (Mead, 2004). It is the relationship between Mb content and its reactions, influenced by muscle pH and temperature, as well as slaughter characteristics and feed that define meat colour and most contribute to the occurrence of poultry meat colour defects (Fletcher, 1999) (table 3).

Table 2 - Myoglobin content in the muscle of several animal species (Sgarbieri, 1996).

Animal Species	Myoglobin concentration (mg/g tissue)
Chicken	0.1
Turkey (*)	Breast muscle – 0.5 Thigh muscle – 2.0
Pork	1.0 – 40
Mutton	6.0 – 12.0
Beef (1 - 2 years)	4.0 – 10.0
Beef (4 - 6 years)	16.0 – 20.0
Whale	50.0

(*) myoglobin concentration values are according to Froning *et al.,* (1978).

Table 3 – Summary of poultry meat colour defects (Fletcher, 1999).

Defects	Description	Possible causes
Bruises and haemorrhages	Classic bruises, pin-point blood spots in meat, blood accumulation along bones and in joints	Physical trauma, nutrient deficiencies, mycotoxins, stunning
Over-scalding	Incomplete removal of epidermis, cooked discoloration on surface of meat	Too high scalding temperature, too long time in scalder
Surface drying	Mottled appearance of skin or meat due to surface dehydration	Incomplete removal of epidermis, (skin), exposed meat, poor packaging, freezing (freezer burn)
Haem reactions	Normal colour ranges from raw pink meat, tan to brown raw meat, grey to brown cooked meat, pink cooked meat, cured meat colour	Oxidative or redox state of the myoglobin, myoglobin complexing with nitrites/nitrates or other compounds such as carbon monoxide
Dark meat	Darker than normal appearing meat, possible mottling	High muscle pH due to ante-mortem depletion of muscle glycogen
Light meat	Pale breast meat	Low muscle pH (PSE-like

		condition)

1.4 Oxidation Promoting Factors

In packaged fresh meats the colour is susceptible to change, according to state of Mb, which can exist in any of the four redox states, namely deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb), and metmyoglobin (MetMb). DeoxyMb, OxyMb, and COMb are in a ferrous state (Suman & Joseph, 2013). OxyMb and COMb provide bright cherry-red colour, critical to consumer acceptance, and the red colour of these two redox forms is indistinguishable by human eyes (Cornforth & Hunt 2008). OxyMb, which forms through exposure to air, saturating Mb with oxygen, is responsible for the red bright colour of meat, being considered the most attractive colour by the consumer (Fletcher, 1999). Mb has greater affinity to CO than to oxygen, resulting in increased stability of bright cherry-red COMb (Suman & Joseph, 2013); this state is not common as it is only seen in the presence of carbon monoxide (CO) gas. DeoxyMb is purplish-red colour; it is the purple pigment of inner muscle and is also seen in vacuum-packaged meat. This colour is recognized as a defect by the consumer (Mancini & Hunt, 2005). MetMb, the oxidized state of Mb, which is grayish-brown because of prolonged exposure of pigments to light, heat, oxygen, certain microorganisms and also low temperatures (freezing temperatures), results from oxidation of the pigment and is associated with meat discoloration; this form is also seen as a defect by the consumer (Suman & Joseph, 2013). Conditions such as low pH levels or high storage temperatures contribute to the formation of MetMb, giving the meat a brownish appearance (Bunsic, 2006). Oxidation of meat products is affected as well by metabolic reactions related to cell membrane disruption as by blood migration due to variable cooling rates (Lyon & Lyon, 2002).

1.4.1 Endogenous Factors

The endogenous factors are the ones intrinsic to the product, having an internal cause or origin and can be measured directly on the product, like temperature, pH, water activity (a_w) and microbial load.

Temperature

Temperature is, perhaps, the most important factor, having a connotation both intrinsic and extrinsic to the product, since it is a fundamental factor to be controlled both internally, on the product itself and externally, along the entire chain as to assure product safety, being

therefore essential to keep low temperatures throughout the entire process. However, at some point, it is more difficult to comply with the stipulated temperatures (3 ± 2ºC), such as in the cutting room and labeling room, where higher temperatures are easily reached (≥10ºC). Most regulatory standards require that poultry and poultry products are cooled rapidly and held at temperatures below 4ºC (Mead, 2004). Temperature is the principal factor affecting the rate of microbial growth and hence the shelf-life of chilled meat (Mead, 2004). High temperatures cause the decrease of product humidity giving rise to texture alterations (surface tends to dry out) and microbial load increase, affecting negatively the quality of meat products.

pH and a_W

pH is a very important factor having a great influence in the colour of fresh meat. The state of heme pigment is largely decided by meat pH and the reaction of heme pigments with other reactants such as O_2 is decided by the state of heme iron (Fermi & Perutz, 1981). At high pH, the iron of heme group is predominantly in ferrous state and at low pH ferrous iron conversion to the ferric state is accelerated (Ahn & Maurer, 1989). According to Buckley *et al.* (1995) oxidation of meat and meat products may be induced or supported by several factors and postmortem processes, among which is: I) pH fall, which contributes to inactivation of reductive enzyme systems and stimulation of acid-catalyzed autoxidation of iron; II) state to iron; III) state of Mb, resulting in accumulation of metmyoglobin (MbFe). Moreover, it was found that these heme species show prooxidative activity at low pH (Baron & Andersen, 2002).

This parameter is also related to drip loss. Differences in pH significantly affect storage and processing quality. Low pH meat is characterized by low water holding capacity and poor technological quality being referred to as pale, soft and exudative (PSE). Meat with high pH, known as dry, firm and dark (DFD), is characterized by poor storage quality resulting from a faster rate of odour production and accelerated microbiological growth (Aslam *et al.*, 2011). Low pH restricts microbial growth, concluding that more acid foods are safer from a microbiological point of view. Meat pH is around 6 (figure 2), and the optimal pH for bacterial development is between 6 and 8, making meat products very liable to contamination if not handled properly (Adams & Moss, 2008).

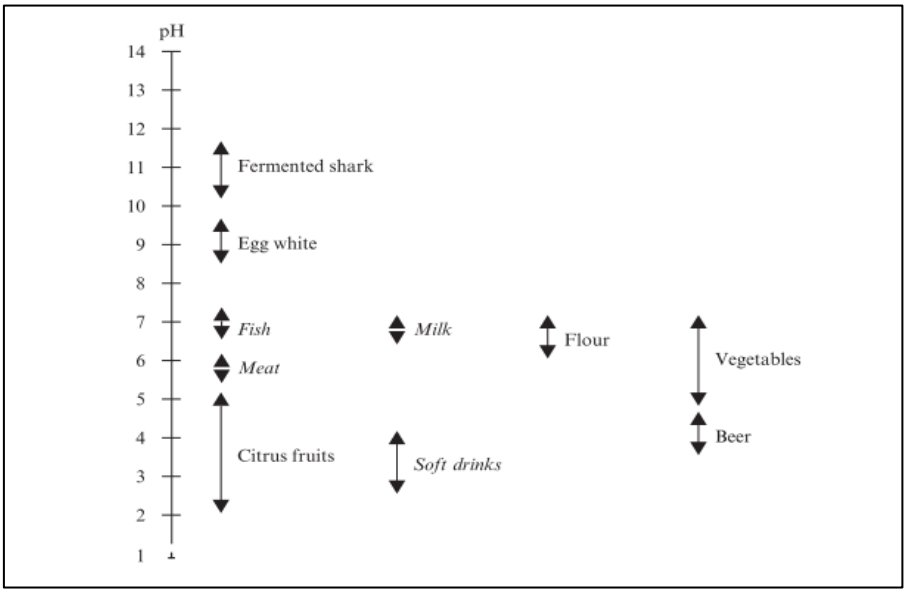

Figure 2 – Approximate pH ranges of some food products (Adams and Moss, 2008).

Water activity is a measure of availability or free water in food. An a_W of less than 0.6 restricts microbial growth and, the higher its value, the greater the presence and development of organisms. Meat has a high a_w value (figure 3), characteristic of a fresh product (perishable), implicating a high risk of microbial development (Adams & Moss, 2008).

According to Jay (2000), in general, most spoilage bacteria do not grow at values below 0.92, spoilage yeasts at below 0.90 and spoilage moulds at below 0.80. Meat, holding an average a_w value above 0.95 is susceptible to all these microbes.

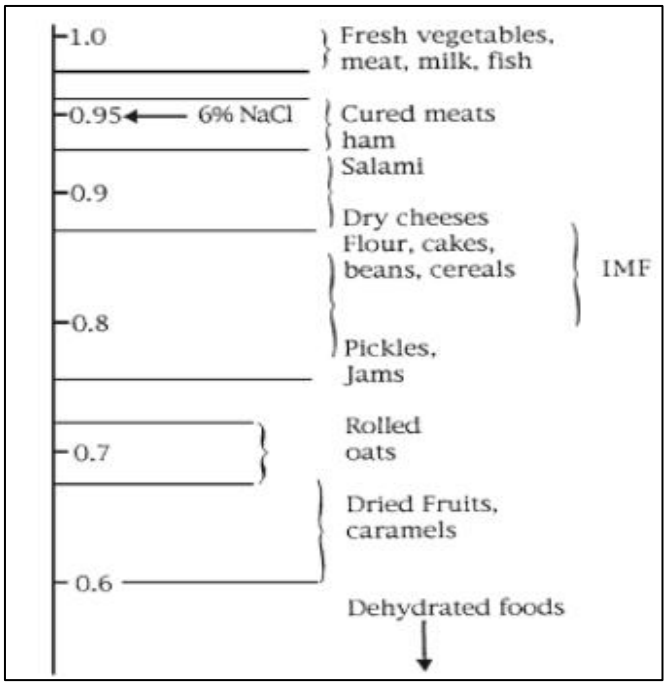

Figure 3 – Range of a_W values associated with several food products (Adams & Moss, 2008).

Packaging atmosphere/meat *ratio*

Many studies have been conducted on the variation of gas composition in MAP, mainly for red meats, pork and beef (Parry, 1993; Jayasingh *et al*., 2002; Seyfert *et al*., 2004). However, there is little research on the effect of gas/meat *ratio*, which plays an important role in product quality. The headspace environment and product behaviour may change during storage in MAP, but there is no additional manipulation of the internal environment (McMillin *et al*., 1999). As stated by Blakistone (1999), headspace gas must be approximately 1.5–2 times the meat volume. A more recent study (Gill & Gill, 2005) reports that package collapse is generally thought to be prevented by headspace gas/meat *ratios* of 2 to 3. Murphy *et al.* (2013) evaluated the effects of gas/meat *ratio* on product quality along shelf-life of beef steaks packaged in MAP with high oxygen content (80% O_2: 20% CO_2). It was concluded that lipid oxidation increased over time in all treatments (gas/meat *ratio* of 2:1, 1:1 and 0.5:1 for 14 days at 4°C). The pH, surface colour, texture and microbial load of beef steaks were not affected. The sensory quality and acceptability of the steaks were similar in the proportion of 2: 1 or 1: 1 but unacceptable at 0.5: 1. This study showed that meat discoloration can be influenced by the relationship between gas and meat on the package, being an important factor to be considered in meat packaging procedures. Another factor to consider, regarding cuvettes with thermosealed lidding film, is the headspace of the package

because product touching the film causes contact discoloration, darkening at a higher rate (McMillin, 2008).

Microbiology

Microorganisms may bring benefits but, in most cases, organisms such as bacteria and *fungi* are seen as a negative factor in food and some are harmful to human health. Unprocessed meat has a microbial load whose composition depends on the way the organisms develop, behave and interact with the food. Most of this microbiota has no consequences on consumers' health because the meat is, generally, processed (cooked) and higher temperatures achieved during cooking process will eliminate or inactivate most of the present microorganisms, pathogenic and/or non-pathogenic. Nevertheless, microorganisms may cause deterioration of organoleptic characteristics, thus causing unpleasant odours (rancid smell) and also affecting food taste and texture. The meat "characteristic" microflora will not cause discoloration but in the right conditions, together with other factors, can contribute, in a first stage to meat maturation and then to deterioration. For instance, while studying the relation of microorganisms and light, Watts (1954) postulated that fresh meats are not materially discolored by display light during a three-day period, but longer display may bring about discoloration primarily due to microbial development.

Microorganisms are important for two reasons. Essentially because they are potentially responsible for ultimate spoilage in those products where microbial growth is favored, as is the case of chilled-stored, raw meat products depending on the numbers of spoilage microorganisms present initially. Secondly, microbial contaminants may include low numbers of particular foodborne pathogens (Mead, 2004). As an alternative to seek a particular pathogen, it is often more appropriate to estimate levels of an appropriate 'indicator' organism instead. The indicators normally used are generic *E. coli*, coliform bacteria and members of the family *Enterobacteriaceae* (Mead, 2007). These indicators, together with some others, are used in the industry at a regular basis to test products; it is a standard food safety requirement in the industry, being a way to control microbiological quality of food products.

The first indication of spoilage in fresh meat is the production of off odours which become relevant when microbial numbers reach around 10^7 cfu/cm^2. Bacterial metabolism produces a complex mixture of volatile esters, alcohols, ketones and sulfur-containing compounds which collectively comprise the off odours detected. The first indication of spoilage is

generally the buttery or cheesy odour, commonly known as rancid, associated with production of diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), 3-methyl-butanol and 2-methylpropanol. These compounds are produced from glucose by members of the *Enterobacteriaceae*, lactic acid bacteria and *Brochothrix thermosphacta* (Adam & Moss, 2008).

1.4.2 Exogenous Factors

The exogenous factors are the ones extrinsic to the product, originating from the surrounding environment and can't be measured directly, like packaging material, packaging atmosphere, light and processing conditions.

Packaging material

The package, as a whole, protects against deteriorative effects (Yam *et al.*, 2005), which may include discoloration, off-flavour and off-odours development, nutrient loss, texture changes, pathogenicity, and other measurable factors (Skibsted *et al.,* 1994).

Packaging materials play a key role in product shelf-life, allowing to preserve and extend the characteristics of the product, delaying its degradation. According to Food and Agriculture Organization (FAO) – "the basic purpose of packaging is to protect meat and meat products from undesirable impacts on quality, including microbiological and physic-chemical alterations. Packaging protects foodstuffs during storage and distribution from:

- contamination by dirt (by contact with surfaces and hands);
- contamination by microorganisms (bacteria, moulds, yeasts);
- contamination by parasites (mainly insects); contamination by toxic substances (chemicals);
- influences affecting colour, smell and taste (off-odour, light, oxygen);
- loss or uptake of moisture (evaporation or water absorption)"

According to the same institution, "packaging materials have certain requirements that need to be fulfilled. Packaging films must be/have:

- flexible;
- mechanical strength;
- light weight;
- odourless;
- hygienic (clean and toxicologically harmless);

- easy recycling;
- resistance to high and low temperatures;
- resistance to oil and fats;
- good barrier properties against gases;
- sealing capability;
- low-cost".

In Portugal, packaging materials are covered by the Regulation (EC) Nº: 2023/2006.

The variables that influence shelf life properties of packaged fresh meat are product, gas mixture, package and headspace, packaging equipment, storage temperature and additives (Hotchkiss, 1989). A very important factor to take in account is the permeability to water vapour (WV) and gases like oxygen and carbon dioxide. When the packaging materials used are only slightly permeable to WV, an increase in WV condensation on the film inner surface will occur, creating better conditions for microbial development (Upmann *et al.*, 2001). The opposite is not recommended as well, as the use of packaging materials with high permeability to WV will cause undesired dehydration. At first, packaging materials, as well as packaging machine, do not present a risk factor, but its characteristics may influence: packaging must be able to keep the moisture content constant within the package, in order to maintain product quality (Stollman *et al*., 1996). In relation to oxygen transmission rate (OTR), Mead (2004) declared that polymer films used for meat packaging can be categorized as: 0±10, low; ~60, medium; and ≥1000, high (measured in cm^3/m^2 in 24h). Films of varying O_2 permeability will affect not only the growth of bacteria, but also the colour and the odour of refrigerated poultry meat (Dawson *et al.*, 1995). According to the same author, generally, low oxygen transmission rate (OTR) films will retard bacterial growth, while high OTR films will reduce the impact of any unpleasant odours when opening the package. Considering the films used in the industry and its OTR, F1= 53.9 cm^3/m^2/24h and F2= 5 cm^3/m^2/24h, it means that F1 has a medium OTR and F2 has a low OTR. Due to this peculiarity, it would be of interest to analyze the effect of packaging material.

Packaging gas composition

There are many studies on the composition of modified atmosphere but mainly focused on red meats (beef and pork), which behave differently from white meats (poultry). Most of the studies indicated that an oxygen-rich atmosphere, around 70/80%, is suitable for fresh meat packaging (table 4), for its advantages in maintaining the appearance and colour of meat (bright red colour), compared to traditional aerobic packaging (McMillin, 2008). On the other

hand, if used in excess, will have negative effects, giving rise to lipid and protein oxidation, appearance of off-odours (such as rancid smell) and contamination by aerobic microorganisms (Lund *et al*, 2007; Skibsted *et al,* 1998). The use of carbon dioxide as a supplementary gas (around 20/30%) balances the negative effects of oxygen through its bacteriostatic properties. As stated by Nassu *et al,* (2010), a concentration of at least 20% inhibits the growth of microorganisms by extending the lag phase and generation time. Carbon dioxide, like all other gases, has its solubility increasing with decreasing temperatures and, therefore, is more effective at lower temperatures. It is water soluble and liposoluble and its absorption by the product originates a reduction of the volume of gas in the package, giving the product a light appearance of vacuum packaging (Kirtil *et al*, 2015) and ultimately may cause package collapse. Packaging containing high concentrations of CO_2 may lead to increased exudate in fresh meat and absorbent pads are usually placed in the package to absorb any excess of liquid.

There are also other studies which defend that the oxygen composition should be limited or even that oxygen should not be introduced into the packages, when referring to white meats, being alternately replaced by inert gas nitrogen (N_2). A study by Saucier *et al*., (2000) using modified atmosphere containing O_2 and a high level of CO_2 (62% CO_2, 8% O_2, and 30% N_2; gas-1), or a gas mixture without O_2 (20% CO_2 and 80% N_2; gas-2) concluded that the colour of ground chicken and turkey meat was more stable in an oxygen-free atmosphere. The use of CO_2 and N_2 extends the growth latency phase of aerobic microorganisms and favors the growth of facultative and anaerobic microorganisms. Thus, an oxygen-free environment may not be the ideal choice as well. However, since anaerobic metabolism produces less intense odours than aerobic metabolism, it is recommended to use a low oxygen concentration in the atmosphere of the package (Rossaint *et al.*, 2006).

Table 4 - Examples of some meat products specifications – gas mixture composition, gas/meat *ratio*, typical shelf-life and storage temperature (Linde AG, 2017).

Product	Gas mixture	Gas volume / Product volume	Typical shelf-life: Air	Typical shelf-life: MAP	Storage temp.
Raw red meat	60–80 % O_2 + 20–40 % CO_2	100–200 ml / 100 g meat	2–4 days	5–8 days	2–3 °C
Raw light poultry	40–100 % CO_2 + 0–60 % N_2	100–200 ml / 100 g meat	4–7 days	16–21 days	2–3 °C
Raw dark poultry	70 % O_2 + 30 % CO_2	100–200 ml / 100 g meat	3–5 days	7–14 days	2–3 °C
Sausages	20–30 % CO_2 + 70–80 % N_2	50–100 ml / 100 g prod.	2–4 days	2–5 weeks	4–6 °C
Sliced cooked meat	30 % CO_2 + 70 % N_2	50–100 ml / 100 g prod.	2–4 days	2–5 weeks	4–6 °C

Light

Light is a factor with direct influence on the product and can have implications since the birth of the animals, influencing their constitution, eating habits and growth (Mead, 2004). It plays an important role in industry along processing but mainly in retail display, where purchase decision occurs. Oxidation of meat pigments is influenced by the levels and wavelengths of light. Bertelsen & Skibsted (1987) concluded that UV radiation contributes more than visible radiation to metMb formation and colour change in meat. However, it should be noted that for a better preservation, meat should not be exposed to any type of light. From a broad point of view, the effects of light exposure may be summarized as the oxidation of fats and oils, the formation of unpleasant off-flavours, losses of vitamins A, B_2, C (Bekbolet, 1990) and, in relation to meat products specifically, it includes elevation of the temperature on meat surface, photochemical effect resulting in greater "destruction" of heme pigments and consequent discoloration of myoglobin (Kropf, 1980).

The radiant energy of the store's walls, ceiling, and floor also can affect product temperature, even when the lights are off. Warmer temperature on the surface of the muscle has the potential to encourage discoloration more quickly (Seyfert *et al.,* 2004).

1.5 Oxidation Prevention Methodologies

The methods used in the past have become basic techniques in the preservation of food and the extension of fresh foods shelf-life. Methods such as refrigeration, proper packaging, and improved processing conditions have been used to delay food deterioration, to hold its organoleptic characteristics (such as appearance, odour and texture) and to prevent safety and quality issues as loss of nutrients and microbial contamination. Huis in't Veld (1996) reported that changes in the extrinsic conditions of the product (for example, refrigeration and MAP), are the only way to delay spoilage. These methods have only been improved over time through investment in a greater know-how, introduction of new equipment and packaging materials, new processing techniques and a deepen knowledge on food quality and safety by operators and engineers.

There are many works already focusing on the prevention of oxidation of meat products. With increasing technological advances, we assist to developments in several areas and referred below are a few examples of new forms of oxidation prevention that are already being implemented or other examples of methods that are not yet in practice:

-In the field of packaging materials with active packaging: divided by oxygen scavengers that maintain oxygen level inside the package at almost 0%, moisture absorbers or absorbent pads used to reduce accumulation of purge from package, antimicrobial packaging acting as carriers of antimicrobial compounds and as barriers to microorganisms (Mead, 2004); intelligent packaging: sensors and biosensors, used to detect, locate or quantify energy or matter giving a signal for the detection or measurement of a physical or chemical property to which the device responds, freshness indicators showing product's quality information, determined by microbial growth or chemical changes within a food product (Biji et al, 2015).

-In the field of Chemistry with the development of solutions with specific characteristics, of natural or artificial origin, that could be ingested with the product. In the specific case of meat, the solution would act like an insulator to the external agents like oxygen, delaying the deterioration of meat and preserving its organoleptic characteristics for a longer time.

-In the field of microbiology, using known antibiotics, such as aureomycin, in order to prolong food characteristics at refrigeration temperatures (Freitas e Figueiredo., 2000).

2. Materials and Methods

The first assay focused on the packaging material, comparing two different films, transparent film, F1 and printed film, F2, with different oxygen transmission rates (OTR), F1=53.9 $cm^3/m^2/24h$ and F2=5 $cm^3/m^2/24h$. The second assay focused on meat proportion, comparing two atmosphere/meat *ratios*, where C1 corresponded to a cuvette with four skewers and an atmosphere/meat *ratio* of 1,16 (C1=1,16) and C2 corresponded to an alternative cuvette with only three skewers and an atmosphere/meat *ratio* of 2,11 (C2=2,11), consequently with less meat/more headspace. The third assay focused on a storage condition, comparing two types of light, L1 stored with light (25 Watt LED light bulb), simulating supermarket display and L2 stored in the dark.

The product was monitored along shelf-life period (7 days at 3 ± 2ºC) and, for all assays, several parameters were measured:

- packaging atmosphere (O_2/CO_2 composition), pH, aW and sensory evaluation (colour, odour, appearance and texture characterization) (these parameters were measured every day during the established shelf life period);
- colour, through the CIE Lab system that measures L*, a*, b* parameters and oxidation stability through TBARS measurements – measured on the first and final day of shelf-life period (D0 and D7);
- microbiological analysis through research and counting of Coliforms (Col), Total Aerobic Mesophiles (Mots), E. coli, Lactic Acid Bacteria (Lac), Yeasts (Lev) and Molds measured on first, third, fifth and final day (D0, D3, D5 and D7) and research of Salmonella spp and L. monocytogenes, measured on first and final day of shelf-life period (D0 and D7).

2.1. Assay 1 – Effect of Packaging Material on turkey meat products

The purpose of this assay was to test the films (printed and non-printed) used in the industry and to measure its impact on the product along shelf-life period. The product – turkey skewers, was followed during its shelf-life period (7 days at 3 ± 2ºC). F1 is the code given to the packages with transparent film and F2 the code given to the packages with printed film.

F1 film is a laminate based on polyethylene (PE), polyethylene terephthalate (PET) and polypropylene (PP) while F2 film is a laminate based on PE, ethylene vinyl alcohol (EVOH) copolymer and polyamide (PA). The difference in constitution between films gave rise to

different behaviour of the product as concluded below, although both films present many similarities as seen on table 5, for thickness, weight, yield, sealing temperature and even present features with same value for both films, as water vapour transmission rate (WVTR) with 13g/m^2/24h. The only feature presenting significant difference between films is oxygen transmission rate (OTR): F1 – 53,9 cm^3/m^2/24h and F2 – 5 cm^3/m^2/24h (F1 value is approximately ten times higher than F2).

Table 5 – Characteristics of films used in the packaging stage (data provided by the company).

Features	Transparent Film (F1)	Printed Film (F2)
Manufacturer	Ronzulli S. p. A.	Bemis Packaging Solutions
Model	Rpack T/E EL PP AF11 52	Opalen HB 55 AF
Material	PET, PP, PE	PA, EVOH, PE
Thickness (mm)	52	55
Weight (per m^2)	56	53
Yield (m^2/kg)	17,85	18,9
O_2TR (cm^3/m^2/24h)	53,9	5
N_2TR (cm^3/m^2/24h)(i)	-	0,9
CO_2TR (cm^3/m^2/24h)(i)	-	25
WVTR (g/m^2/24h)	13	13
Sealing Temperature (ºC)	130-150	120-140

(i) - No data was provided for this parameter.

2.2. Assay 2 – Effect of Atmosphere/Meat Ratio on turkey meat products

The objective of this assay was to compare packages with different atmosphere/meat *ratios*, along shelf-life period (7 days at 3 ± 2ºC). Two different packages were analyzed, one with four skewers (C1) and an atmosphere/meat *ratio* of 1,16 (table 6) and another package (C2) with three skewers only and consequent atmosphere/meat *ratio* of 2,11 (table 7). This way the headspace impact was considered, based on the packaging atmosphere, quantity of meat per package and the way these two indicators interacted. The objective of this assay was to evaluate the influence of the available protective atmosphere on product colour (promotion or delay of discoloration).

The package used on this assay had the same characteristics of the package evaluated on assay 1 – F1: transparent film with OTR=53,9 cm^3/m^2/24h based on polyethylene (PE), polyethylene terephthalate (PET) and polypropylene (PP).

Table 6 - Calculation of the atmosphere/meat *ratio* for C1.

Product	Weight (cuvette + skewers) kg	Weight (cuvette + skewers + water) kg	Weight (water:atmosphere) kg	Weight (cuvette) kg	Weight (skewers) kg	*Ratio* (atmosphere/meat)
C1	0,662	1,416	0,754	0,0147	0,647	0,754/0,647=1,16

Table 7 - Calculation of the atmosphere/meat *ratio* for C2.

Product	Weight (cuvette + skewers) g	Weight (cuvette + skewers + water) kg	Weight (water:atmosphere) kg	Weight (cuvette) kg	Weight (skewers) kg	*Ratio* (atmosphere/meat)
C2	0,464	1,415	0,951	0,0147	0,150x3=0,450[(I)]	0,951/0,450=2,11

[(I)]Each package takes four skewers and the skewers net weight is ~0,600 kg and consequently one skewer net weight is ~0,150 kg. Package C2 only takes 3 skewers, totalizing ~0,450 kg.

2.3. Assay 3 – Effect of Light on turkey meat products

The purpose of this assay was to analyze the influence of light exposure on the final product along shelf-life period (7 days at 3 ± 2ºC). Turkey skewers were exposed to different types of light to investigate the influence of light. The product was stored in the refrigeration room with standard light conditions, simulating retail display (L1). L2 were stored at similar temperature conditions, but in the absence of light.

Characteristics of the light used in the refrigeration room (L1):

- Denomination: LED Tube T8 25W - 1500mm Frosted 6000K
- Wattage: 25W;
- Voltage: AC100-240V;
- Type of LED: SMD (surface mounted device);
- Light beam angle: 120º (degrees);
- Luminous Flux: 2450lm;
- Light Colour: 6000K;
- Cover: frosted plastic;
- Dimensions of each light structure – length: 1500mm; width: 26mm.

The package used on this assay had the same characteristics of the package evaluated on assay 1 – F1: transparent film with OTR=53,9 $cm^3/m^2/24h$, based on polyethylene (PE), polyethylene terephthalate (PET) and polypropylene (PP).

2.4 Turkey meat products processing technology

A flowchart with common steps to all turkey fresh meat products processing is presented on annex 2. The process is divided in three main areas: reception, production and expedition.

Technological process of turkey skewers is described as follows. A specific flowchart of turkey skewers processing can be seen on annex 3. Raw materials (RM) used for TS have two different sources: national suppliers (from slaughterhouses) and imported (Spain, Italy and Poland). Origin was a conditioner of the process because each meat had its own specifications and way of packaging: RM from national sources were supplied as refrigerated carcasses to be deboned at the industry and the imported meat was supplied already processed, vacuum packaged refrigerated or frozen turkey leg. From the carcass only the thigh was of interest for the skewers production. The meat was processed altogether with green peppers (ELS, Portugal) and bacon (Paranho Carnes, Portugal) by an operator and sliced on a cutting machine (Bucelmaq, Portugal). The TS were packaged under MAP; the packaging was constituted of EPS cuvettes (Coopbox, Italia) with absorbent pads, wooden sticks (Begal, Portugal), thermo-sealable laminate (Ronzulli, SpA, Italia; Bemis Packaging Solutions, USA). Skewers were packaged in a horizontal wrapping machine (Mondini Proface, p. A, Italy) and the protective atmosphere applied in this study was 70% O_2:30% CO_2 (Air Liquide, France). After packaging, the samples were stored in the refrigeration room maintained at 3 ± 2 ºC, until expedition order.

2.5 Experimental Procedure

For the analysis of the product the following aspects were considered:

- Provenience of meat: national or imported;
- Type of packaging and type of film used;
- Type of atmosphere: protective atmosphere (70% O_2:30% CO_2);
- Ingredients: meat, bacon and peppers;
- The product analyzed in each assay came from the same batch and was processed, collected and packaged at the same time;
- Each experiment was performed independently and procedure was the same for all assays. The assays were performed following a strict order described below:

Tightness Test

The tightness test was carried out after packaging at all samples to check for any defects like poor sealing and pinhole punctures that may occur at the time of packaging. The test was performed by dipping the packages into a container of sufficient size full of water so that it fitted completely submerged, and deformations and leaks were detected through release of bubbles from the package.

Microbial Analysis

Samples were analyzed at day 0, 3, 5 and 7 (day 0 corresponding to day of packaging). On each sampling day, three samples were analyzed (n=3). In the industry laboratory was performed, according to Regulation (EC) nº 1441/2007, coliforms, total mesophilic aerobic microorganisms, lactic acid bacteria, moulds, yeasts and *Escherichia coli* determinations. External analysis was also performed at one accredited laboratory for *Salmonella spp* and *Listeria monocytogenes* detection and enumeration.

The preparation of the samples was carried out in accordance with ISO 6887-2. The internal analysis proceeded by aseptically remove 10g of sample to a sterile Stomacher Bag (Seward, UK) containing 90ml of tryptone salt (0.1% w/v) (Oxoid, England) and blend in the stomacher during 30 seconds. The 10g of sample were composed of thigh meat, pepper and bacon portions, taken from different sections of the skewers, obtaining a homogeneous and representative mixture.

Preparation of dilutions (NP 3005:1985): Decimal dilutions were performed according to the technique described in NP 3005:1985. Thus, 1 ml of the initial suspension was withdrawn and transferred to a test tube containing 9 ml of tryptone salt obtaining the 10^{-2} dilution and homogenizing in the vortex. This was done successively up to the dilutions deemed necessary.

Performed analysis

Tenfold dilutions in tryptone salt were spread plated on the following culture media. Mesophilic counts were made on plate count agar (PCA) (Biokar Diagnostics, France) and incubated at 30ºC for 3 days, (ISO 4833-1: 2013). Lactic acid bacteria counts were determined by sample incorporation on Man, Rogosa and Sharpe agar (MRS) (Biokar Diagnostics, France), incubated at 30ºC for 3 days (ISO 15214: 1998). Yeasts and moulds were enumerated on Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) (Oxoid,

UK), and incubated for 5 days at 25ºC (ISO 21527-1: (2008). Coliforms counts were determined by sample incorporation on Violet Red Bile Lactose Agar (VRBL Agar) (Oxoid, UK), incubated at 37ºC for 24h (ISO 21528-2: 2014). *E. coli* counts were determined by sample incorporation on Tryptone Bile X-Glucuronide Agar (TBX Agar) (Merck, USA), incubated at 44ºC for 24h (ISO 16649-1: 2001). All microbiological analysis were carried out in triplicate (n=3) and the results were converted to a logarithm of the number of colony-forming units (CFU/g). The external analyses were also carried out according to the respective regulations to each procedure: Horizontal method for the detection of *Salmonella spp*. (ISO 6579: 2002) and Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria spp*. (ISO 11290-1: 1996 – Part 1: Detection method).

Measurement of atmospheric composition

Measurement of the atmospheric composition (% O_2 and % CO_2) was performed along shelf-life period of seven days with a gas meter (Checkpoint O_2 / CO_2 meter, Dansensor, Denmark). Atmospheric composition was measured daily on one sample of each package and the results were expressed in gas percentage.

Sensory Evaluation

Sensory evaluation was performed along shelf-life period of seven days and consisted on the evaluation and description of organoleptic characteristics (colour, smell, texture) and consequent photographic recording. Sensory attributes were measured daily on one package of each kind.

Measurement of pH

It was measured along shelf-life period of seven days. Determination of pH was done with a meat pH meter, glass electrode (HANNA Instruments, USA). pH was measured daily on three samples of each package.

Measurement of a_W

It was measured along shelf-life period of seven days. Determination of a_W was done in meat, bacon and pepper pieces with a meat a_W meter (Hygropalm - HP23-AW-A, Rotronics, France) to all samples. a_W was measured daily on three samples of each package. Both pH and aW, as well as atmospheric composition and sensory evaluation were measured at room temperature (approx. 25ºC).

Measurement of Temperature

The temperature was measured throughout shelf-life period of seven days, to control the temperature of the samples in the refrigeration room. The measurement was done by penetration with a thermometer (Etiltd, UK) to all packages after measurement of atmospheric composition. Temperature was measured daily on two samples of each package.

Measurement of Colour

These parameters were measured in the first and last sampling days (day 0 and day 7). The colour was measured with a colorimeter (Chroma Meter CR-400, Konica Minolta, Japan) (n=3), with the CIE L*a*b* system, where L* is a measure of the lightness (0 being the darkest and 100 being the lightest), a* is a measure of redness, ranging between green (-values) and red (+values), and b* is a measure of yellowness, varying between blue (-values) and yellow (+values). All colour measurements were carried out ten times, at different sites of the skewers to obtain a more homogeneous overall result.

Measurement of TBARS

Thiobarbituric Acid Reactive Substances (TBARS) are an indication of lipid oxidation of a sample. The TBARS value should increase as the extent of lipid oxidation increases in a sample. TBARS were measured following a Demiral & Turkan (2005) adapted protocol (n=3):

On the first day there was no distinction between the packages since it was the day of packaging and there weren't relevant differences yet, and at the last day there were two different packages analysis; All TBARS measurements were carried out in triplicate.

The analysis proceeded by aseptically taking a portion of meat from the package and grinding it in a meat grinder (Northern Tool + Equipment, USA) removing 0,5 g of sample to a mortar containing 2,5 mL de 0,1 % (p/v) TCA (0.1% solution of trichloroacetic acid in deionized water). With a pestle the mixture was homogenized as best as possible. 1500 µL were then centrifuged at 12 000 x g (Sigma-Aldrich, USA) during 15 minutes. After that 1 mL of supernatant was mixed with 4 mL of 20% TCA + 0.5% TBA solution (20% solution trichloroacetic acid and 0,5% thiobarbituric acid in deionized water) proceeding to heat at 100ºC for 30 minutes followed by rapidly cooling in an ice bath to stop the reaction. After a few minutes the solution was centrifuged at 10,000 x g for 15 minutes finishing with absorbance reading in a spectrophotometer (Analytik Jena, Germany) at 532 nm and at 600

nm in glass cuvettes. The results were expressed in millimolar (mM) of malondialdehyde (MDA).

2.6 Statistical Analysis

After evaluating the parameters and recording the results, the data was analyzed. The results of the parameters measured daily (O_2, CO_2, pH and a_W) were presented in graphs and statistical analysis was performed using daily mean values of each sample, using the software R (a software environment for statistical computing and graphics). Differences between mean values were evaluated by a *t*-test to verify the difference of mean values between samples (p-value > 0.05) and the assumptions of normality were verified using the Shapiro-Wilk test (p-value > 0.05).

For the results of the parameters colour, TBARS and microbiology it was not possible to proceed with statistical analysis because the number of repetitions was insufficient and, consequently, remaining parameters results were only organized and compared in graphs, for microorganisms and in tables, for colour and TBARS. In graphs, results were presented using mean values and in the tables all data were presented as mean ± standard deviation ($\mu \pm \sigma$). The sensory evaluation was a simple descriptive evaluation, as mentioned above, considering appearance, colour, odour and texture.

3. Results and Discussion

Starting by packaging atmosphere analysis and the evolution of O_2 and CO_2 gases along product shelf-life, an expected decrease tendency was observed for O_2 gas and an increase tendency was observed for CO_2 gas.

The established initial O_2 percentage value for modified atmosphere packaging was 70% and the average value on day 7 was 59,1%. Measured values varied from 76,4% for L2 on day 2 and 53,4% for C1 on day 7 (figure 4). A similar trend was noted for C and L while F presented higher average values and a different behaviour on the final days – F2 starting to increase on day 4 and F1 starting to increase on day 6.

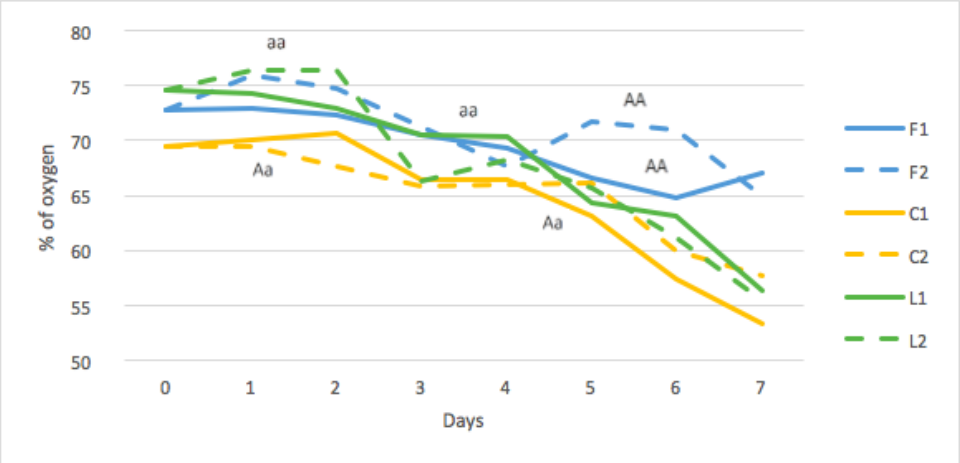

Figure 4 – Evolution of O_2 (%) along product shelf-life (7 days at 3 ± 2ºC). F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; C referred to atmosphere/meat *ratios*: C1 with lower *ratio* and C2 with higher *ratio*; L1 storaged with light and L2 storaged without light. For each assay same pair of letters means that results do not differ significantly (*p*-value>0.05); Assay F (uppercase letters); Assay C (upper and lowercase letters); Assay L (lowercase letters).

For CO_2, the established initial CO_2 percentage was 30% and the average value on day 7 was 32,4%. Measured values oscillated between 37,8 for F2 on day 4 and 17,6 for L1 on day 1 (figure 5). Once again C and L presented almost identical behaviour while F displayed higher average values and a different tendency on the last days – F2 showed abnormal behaviour, presenting an irregular decrease of CO_2%.

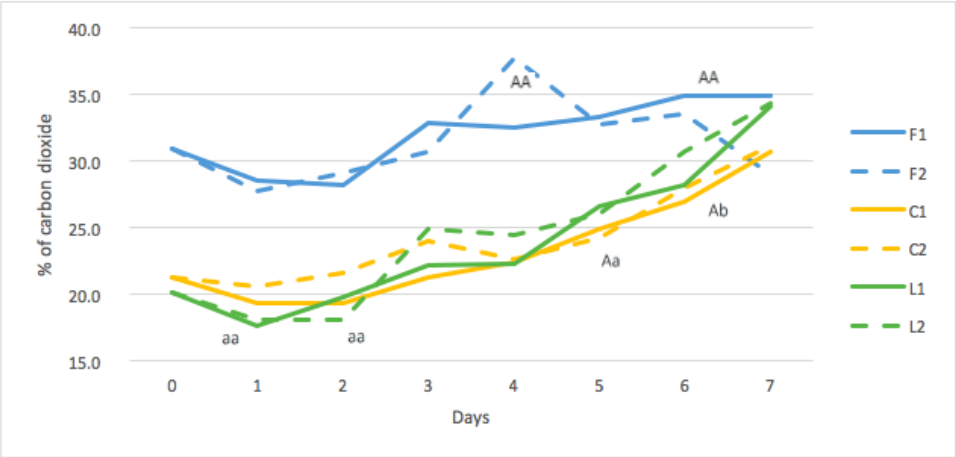

Figure 5 – Evolution of CO_2 (%) along product shelf-life (7 days at 3 ± 2ºC). F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; C referred to atmosphere/meat *ratio*s: C1 with lower *ratio* and C2 with higher *ratio*; L1 storaged with light and L2 storaged without light. For each assay same pair of letters means that results do not differ significantly (*p*-value>0.05); Assay F (uppercase letters); Assay C (upper and lowercase letters); Assay L (lowercase letters).

Initial meat pH value was ± 6.4 (slightly acidic), the average value on day 7 was 6,1 and measured values ranged from 6,5 for F1 on day 0 and 5,9 for C2 on day 6 (figure 6). This parameter showed a similar trend along shelf-life period (7 days at 3 ± 2ºC), for all assays.

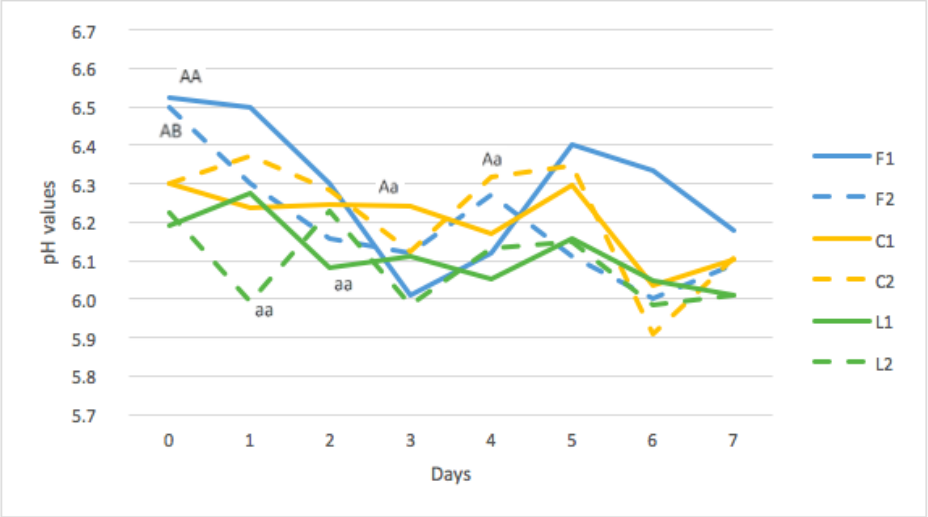

Figure 6 – Evolution of pH along product shelf-life (7 days at 3 ± 2ºC). F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; C referred to atmosphere/meat *ratio*s: C1 with lower *ratio* and C2 with higher *ratio*; L1 storaged with light and L2 storaged without light. For each assay same pair of letters means that results do not differ significantly (*p*-value>0.05); Assay F (uppercase letters); Assay C (upper and lowercase letters); Assay L (lowercase letters).

The established a_W value for meat products is around 0,995 (Adams & Moss, 2008) and the initial average value was 0,709. Final average value was 0,694 and measured values ranged from 0,789 to F1 on day 6 and 0,647 for C1 on day 5 and C2 on day 5 and 6 (figure 7). The obtained values were substantially lower than the stablished meat a_w value and it may be due to the fact that measurements were executed with meat portions exposed to the elements (implying a loss of humidity on the process) and that the measurement included a homogeneous portion of the product, including bacon and pepper that ultimately influenced obtained values.

Obtained results of pH and a_W within assays differed in a very short range, suggesting that this variation is not relevant and that these parameters were not so influenced by the variables studied – packaging material (F), atmosphere/meat *ratio* (C) and light conditions during storage (L).

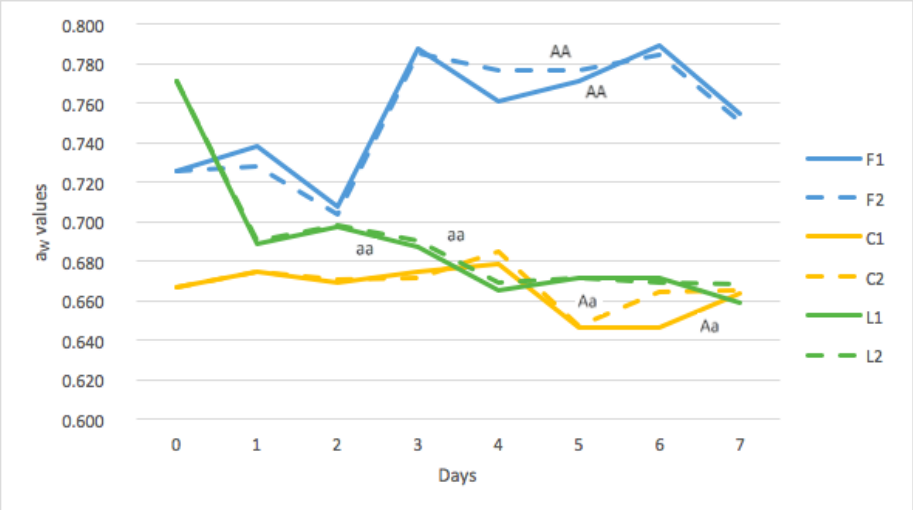

Figure 7 – Evolution of a_W along product shelf-life (7 days at 3 ± 2ºC). F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; C referred to atmosphere/meat *ratios*: C1 with lower *ratio* and C2 with higher *ratio*; L1 storaged with light and L2 storaged without light. For each assay same pair of letters means that results do not differ significantly (*p*-value>0.05); Assay F (uppercase letters); Assay C (upper and lowercase letters); Assay L (lowercase letters).

Table 8 – Colour (n=10, $\bar{x}\pm SD$) and TBARS (nmol MDA/g) (n=3, $\bar{x}\pm SD$) measured values along product shelf-life (7 days at 3 ± 2ºC). F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; C referred to atmosphere/meat *ratio*s: C1 with lower *ratio* and C2 with higher *ratio*; L1 storaged with light and L2 storaged without light.

Day	Colour						TBARS	
	L		a		b			
Assay 1								
0	46,10 ± 4,13		15,59 ± 2,97		9,47 ± 2,11		0,431 ± 0,236	
	F1	F2	F1	F2	F1	F2	F1	F2
7	48,61 ± 3,21	50,08 ± 3,44	14,82 ± 4,97	12,74 ± 5,54	10,64 ± 1,97	10,32 ± 1,96	1,137 ± 0,309	1,533 ± 0,033
Assay 2								
0	45,46 ± 3,76		15,18 ± 4,03		8,99 ± 2,55		0,168 ± 0,032	
	C1	C2	C1	C2	C1	C2	C1	C2
7	48,55 ± 4,61	49,16 ± 5,70	13,00 ± 4,11	16,33 ± 2,55	10,12 ± 2,84	10,31 ± 1,82	1,339 ± 0,655	0,468 ± 0,036
Assay 3								
0	44,28 ± 2,74		14,00 ± 2,75		6,13 ± 1,91		0,061 ± 0,018	
	L1	L2	L1	L2	L1	L2	L1	L2
7	47,74 ± 2,52	47,89 ± 3,74	11,38 ± 4,41	13,37 ± 3,68	5,35 ± 1,08	6,19 ± 2,41	0,657 ± 0,027	1,158 ± 0,753

Colour and TBARS were both analysed only on day 0 and day 7 (table 8). Each assay had results for colour parameters lightness, redness and yellowness (L*, a* and b* respectively) and also TBARS; all data was presented as means with relative standard deviations (μ+σ).

It was hypothesized that as lipid oxidation increased, so would the extent of L* and b* values while a* values decreased, observing a lighter colour of the meat, while also reducing redness and increasing yellowness, which suggests colour deteriorates over time as oxidation occurs causing a negative impact. The three assays held true to this trend, except a^*_{C2} and b^*_{L1} results.

Assay 1 was the only one in which both packages followed the hypothesis, contrarily to assays 2 and 3. F2 showed higher variations in all parameters except in b* (b_{Fd0}= 9,47 ± 2,11; b_{F1d7}=10,64 ± 1,97 and b_{F2d7}=10,32 ± 1,96), suggesting that packaging material had no substantial effect in yellowness variation. In assay 2, results were not consistent – C2 demonstrated a higher increase in lightness and yellowness and a peculiar increase in

redness; C1 lipid oxidation values were approximately three times C2 ($TBARS_{C1}$= 1,339 ± 0,655; $TBARS_{C1}$= 0,468 ± 0,036). Assay 3 was characterized by having lower values for all parameters, comparing to assay 1 and 2; both packages showed very similar lightness results. L1 had the lowest redness value (a^*_{L1d7}= 11,38 ± 4,41) and presented a contradictory decrease in yellowness while L2 presented a small increase. As for lipid oxidation results, day 0 value was very low and L2 displayed a higher increase for day 7.

Microbiology results were expressed in bar charts (figures 8, 9, 10); there was one chart for each assay and each chart showed values obtained for coliforms, mesophilic aerobic bacteria, lactic acid bacteria and yeasts, which were the microorganisms that displayed the most significant results. Numbers for *E. coli* and moulds were residual and consequently their results were not expressed.

Each figure showed four pairs of bars representing the results of the microbiological analysis. Each set of bars was represented by a specific colour. Sample one was represented by a paler hue and sample two by a darker hue. For microorganism 1 (coliforms) the chosen colour was blue, for microorganism 2 (total plate count at 30ºC) yellow, for microorganism 3 (lactic acid bacteria) green and for microorganism 4 (yeasts) brown. Each microorganism had a code name followed by the number of the sample (1 or 2): for coliforms it was “coli”; for mesophilic aerobic bacteria it was “mots”; for lactic acid bacteria it was “lac”; and for yeasts it was “lev”.

In all graphics the microbial counts were expressed in log CFU/g (logarithm of colony forming units existing in one gram of sample). In figure 9, coliforms count for both samples on day 0, and also yeasts counts for L1 on day 7 were not presented; as well as coliforms counts for F1 on day 0 (figure 10).

Assay 1 showed high numbers of mesophilic aerobic bacteria and also of lactic acid bacteria (figure 8). Assay 2 showed high numbers of coliforms and yeasts with C1 presenting increasing number of yeasts along product shelf-life and C2 higher numbers of coliforms (figure 9). Assay 3 revealed a high number of coliforms and mesophilic aerobic bacteria and package L2 generally presented more counts (figure 10).

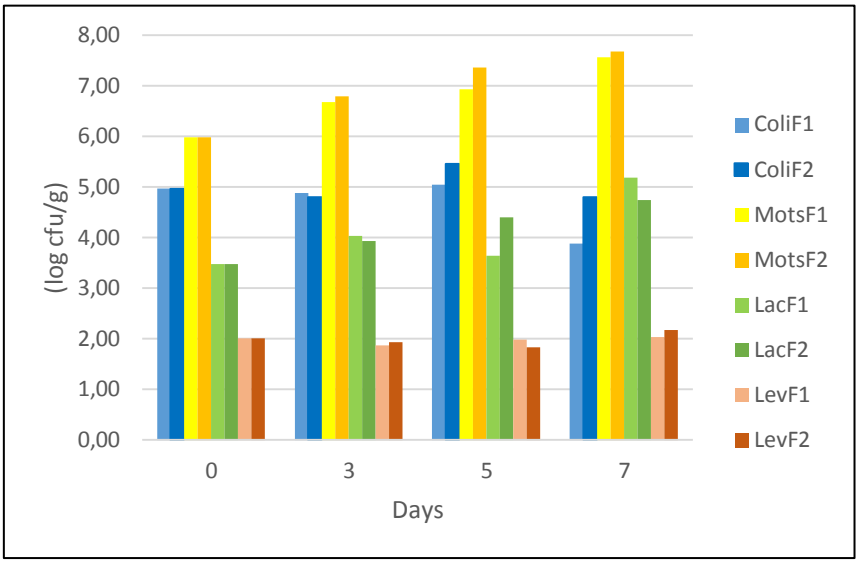

Figure 8 – Microbiological analysis related to packaging material influence. F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR. Coliform bacteria (coli), total mesophilic aerobic microorganisms (mots), lactic acid bacteria (lac) and yeasts (lev) counts (log cfu/g) along product shelf-life (7 days at 3 ± 2ºC).

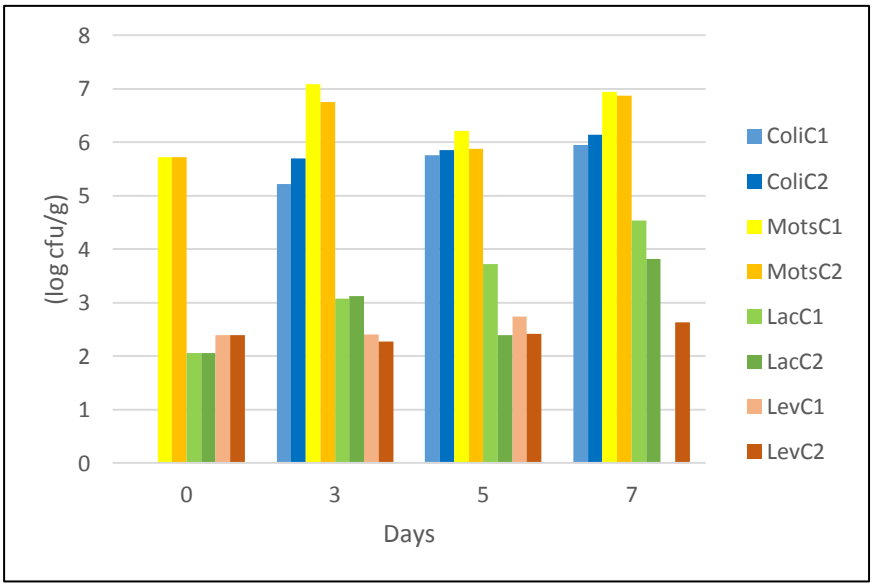

Figure 9 – Microbiological analysis related to atmosphere/meat *ratio* effect. C referred to atmosphere/meat *ratios*: C1 with lower *ratio* and C2 with higher *ratio*; Coliform bacteria (coli), total mesophilic aerobic microorganisms (mots), lactic acid bacteria (lac) and yeasts (lev) counts (log cfu/g) along product shelf-life (7 days at 3 ± 2ºC).

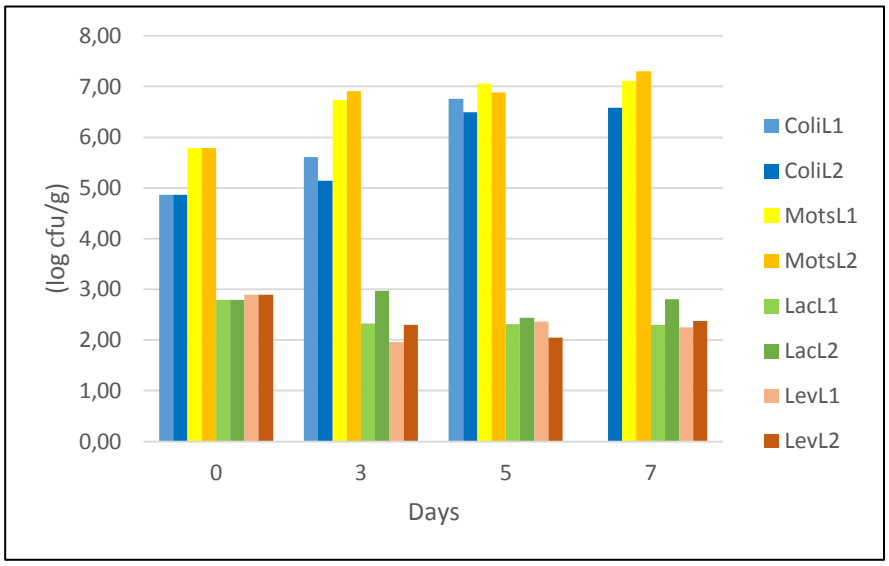

Figure 10 – Microbiological analysis related to storage light conditions. L1 storaged with light and L2 storaged without light. Coliform bacteria (coli), total mesophilic aerobic microorganisms (mots), lactic acid bacteria (lac) and yeasts (lev) counts (log cfu/g) along product shelf-life (7 days at 3 ± 2ºC).

Due to the relevance of the results obtained, it was therefore necessary to highlight the first assay and the variable studied, evidencing the impacts it had on the product and on the parameters along shelf-life period (7 days at 3 ± 2ºC).

Regarding the parameters analyzed some conclusions were common to all assays: for atmosphere composition results, both gases showed matching results, for instance, a decrease in O_2 concentration was accompanied by a CO_2 increase over time, as it would be expected; the small decrease in CO_2 observed on the first days was expected and was due to the absorption of this gas by the meat. This variation, which was accompanied by O_2 increase, was observed at all samples. pH and a_W were the parameters that demonstrated less pronounced variation along shelf-life period; pH ranged in an extension of values of approximately half point between the maximum (pH=6,5) and minimum value (pH=6,0) and the general results tendency was to decrease along product shelf-life period, translating into an acider pH with time. a_W showed different behaviour between assays but in each assay, both samples generally displayed identical results, with some tendency to decrease along product shelf-life period. Although the tendency was to decrease, assay 1 results indicated otherwise, with both samples showing an irregular increase (figure 7). A hypothetical relationship between colour and TBARS measurements, which was expected, was observed. Samples with increasing values of TBARS revealed some relation to increasing values of L* and b* and decreasing values of a* colour parameters (table 10).

3.1 Effect of Film Type

Package with higher OTR (53,9 $cm^3/m^2/24h$) and transparent film, F1, revealed a more constant rate of variation of O_2 and CO_2, along product shelf-life (7 days at 3 ± 2ºC), while package with lower OTR (5 $cm^3/m^2/24h$) and printed film, F2, presented an irregular rate of variation of both gases, which can be seen, for instance on the increase of O_2 and corresponded decrease of CO_2 from day 4 to day 5 (figure 4 and 5). However, differences between samples were not significant, according to the *t*-test for the difference of mean values. For pH, was observed a high decrease for F1 (approximately 0.5) from the first day to the third and, for F2, the variation was not so accentuated. The behaviour of samples was also irregular in the final days. For F2, pH values differed significantly from each other (*p*-value < 0,05) along shelf-life period. For a_W, both packages (F1 and F2) showed similar behaviour along shelf-life period and according to the *t*-test there was no differences between them.

Concerning colour and TBARS measurements, F2 packages presented a greater increase for L* and TBARS, although for TBARS this increase was greatly affected by relative standard deviation values. For parameter a*, F2 presented a greater decrease. In conclusion, F2 turned more bright, lost more redness, gained almost the same yellowness as F1 and more malondialdehyde (MDA) was formed.

The sensory evaluation corroborates perfectly with the results above, with F2 starting to show discoloration already at day 2. Odour alteration began at day 4 with greater incidence on F2, with unpleasant rancid smell. On day 6, the deterioration signs were obvious, both at odour and colour levels with a serious impact on product organoleptic characteristics.

Microbiological analysis showed, at early stages, contamination for both F1 and F2, with high number of mots (6 log cfu/g) and coliforms (5 log cfu/g), predominantly; lactic acid bacteria showed an increase along shelf-life, leading to an acidification, which was confirmed by pH results. Dawson et al., (1995) stated that low OTR films retard bacterial growth but this analysis revealed similar results for both F1 and F2, thus concluding that the type of film won´t have relevant influence in microbial development along product shelf-life period.

Regarding *Listeria monocytogenes* and *Salmonella* spp, day 0 analysis scored positive on *Listeria* (<1x10^1 cfu/g, which is acceptable) and negative on *Salmonella;* on day 7, F1 scored positive on *Listeria* (<1x10^1 cfu/g, which is acceptable) and negative on *Salmonella*, while F2 scored negative for both.

According to the results, this assay revealed a clear distinction between F1 and F2 for colour, TBARS and sensory evaluation parameters, showing less degradation for F1 along shelf-life period.

3.2 Effect of Atmosphere/Meat Ratio

Assay 2 measurements showed interesting results along shelf-life period. C1 was the standard industrial atmosphere/meat *ratio* of 1,16 and C2 was the atmosphere/meat *ratio* of 2,11. By reducing meat amount on the package a specific trend was expected: lower O_2 consumption (lower respiratory rate), lower colour degradation, less oxidation by-products formation and lower microbial growth, but the results turned out differently.

Packaging gases values varied similarly over time, although CO_2 measurements were significantly different (*p*-value < 0,05) (figure 5). O_2 measurements were higher for C2, as expected, and it started to show on day 4 (figure 4). In relation to pH, C2 showed more pronounced oscillations but both samples ended up with approximated values. For a_W both C1 and C2 showed similar behaviour during shelf-life period, indicating that a_W was not affected in this assay. This conclusion was based on a *t*-test for the difference of mean values of a_W (p-value > 0.05); the assumptions of normality were verified using the Shapiro-Wilk test (p-value = 0.0494).

Relatively to colour and TBARS measurements, C2 evidenced more brightness, increase in redness and increase in yellowness but, on the other hand, was C1 who displayed greater formation of by-products from lipid oxidation. Colour and TBARS mean values and relative standard deviation values shouldn't be neglected (e.g.: a* result: a^*_{d0}= 15,18 ± 4,03 and a^*_{d7}C2= 16,33 ± 2,55).

As for sensory evaluation, packages began to show discoloration at day 3 and 4. A more rapid deterioration, both on colour and odour of sample C1 was recorded. Although some exceptions, in general, C2 were better looking, more vivid (as seen by colour measurement) and had less intense odour than C1. On day 5 we could clearly detect signs of discoloration and off-odours in both packages. At the end of shelf-life period C1 was more discoloured and had more intense rancid smell.

Microbiological analysis showed that C1 were generally more contaminated, with exception of coliforms. Thus, C2 atmosphere/meat *ratio* had more influence on lactic acid bacteria growth. *Listeria* and *Salmonella* scored negative for day 0 and for C1 on day 7; C2 was not analysed.

This assay showed that C2, with higher atmosphere/meat *ratio,* held its characteristics for a longer period, as it would be expected, despite some contradictory results (for example for colour measurements).

3.3. Effect of Light

For this assay, measurements generally presented similar behaviour along shelf-life period and samples had slight differences. Assay results were not totally in agreement with Bertelsen & Skibsted (1987) which stated that meat products should be preserved in the dark, since packages stored without light (L2) did not obtained notably better results than those stored with light (L1).

Packaging gases measurements were not significantly different. There was, nevertheless, an accentuated gas variation for L2 from day 2 to day 3, as observed on figure 4 and 5. Concerning pH some differences were observed on the first days but L1 and L2 were not significantly different. Regarding a_W (figure 8), L1 and L2 had identical behaviour, but it couldn't be stated statistically, since the assumptions of normality were not valid, based on the Shapiro-Wilk test result.

Regarding TBARS measurements, both samples showed pronounced TBARS increase with L2 presenting a greater increase ($L1_{TBARSd7}$= 0,657 and $L2_{TBARSd7}$= 1,158) and also a higher relative standard deviation value ($L1_{\sigma d7}$= 0,027 and $L2_{\sigma d7}$= 0,753), which would relativize the true difference between samples. Colour measurements evidenced unconformities between packages and the relative standard deviation values adjacent to each measurement shouldn't be neglected. Both L1 and L2 ended up with almost the same brightness, higher decrease in redness for L1, peculiar decrease in yellowness for L1 and greater TBARS increase for L2.

Throughout sensory evaluation L1 displayed the reddest meat and more intense odour in general. Samples began to show discoloration at day 4 and L1 showed faster deterioration than L2. On day 5 the signs of oxidation on both packages were obvious, including characteristic rancid smell, which increased until day 7.

Microbiological analysis showed a greater increase of coliforms for L2 and as for L1 the same can't be affirmed by insufficient data but an increase along shelf-life period was the tendency detected; lactic acid bacteria and yeasts numbers decreased with time. The external microbiological analysis showed satisfactory results, with no positive scores for *Listeria* and *Salmonella,* both on day 0 as for the two samples on day 7.

4. Conclusions

The purpose of this thesis was to study the promoting factors of turkey meat products oxidation, focused on processing industry environment. From the main topic, meat discoloration and the research that followed, four proposals were submitted: (1) packaging material, (2) atmosphere/meat *ratio*, (3) type of storage light and (4) packaging atmosphere. Of the four proposals, three assays were carried out and the assay focusing on protective atmosphere was not carried out, being nevertheless an interesting proposal to consider in future studies. The assays focused on three factors, which were thoroughly studied: packaging material, atmosphere/meat *ratio* and storage light conditions. Some interesting results were obtained for all assays. Assays 2 and 3 were presented first and assay 1 was discussed further in more detail.

For assay 2, atmosphere/meat *ratio*, a faster degradation was expected for C1 (with lower atmosphere/meat *ratio*), implicating a faster depletion of oxygen. Assay observed that both C1 and C2 showed similar results and the differences between them were not relevant. On assay 3, allusive to storage light, results didn't agree with other studies (Bertelsen & Skibsted, 1987), concluding that there is no benefit from a no light storage environment, as the package storaged in the dark (L2) reported worst results in the evaluated parameters. In the experimental conditions mentioned above, no expressive differences were observed between samples, both for assay 2, atmosphere/meat *ratio*, and for assay 3, storage light conditions, which allowed to affirm that assay 2 and assay 3 had little relevance in the discoloration phenomenon of the studied product (turkey skewers), under the conditions tested, because both packages evidenced discoloration before the end of shelf-life period, in both assays.

The first assay, focused on the packaging material, showed relevant differences between packages. According to the previous research (Dawson *et al*., 1995), it would be expected a slower degradation of sample F2 (package with lower OTR) but the results showed otherwise, with F1 holding its characteristics for longer. The type of film was the variable with more influence: it showed greater discrepancy between samples in daily measurements (O_2, CO_2, pH, a_W); with respect to colour and TBARS, it was the only assay whose results were in conformity with the proposed hypothesis between colour and TBARS. It was the only one showing a clear distinction in sensory evaluation with F2 presenting increased odour degradation along product shelf-life (7 days at 3 ± 2ºC) and F1 presenting milder odour degradation, being in agreement with the study of Dawson *et al*., (1995), which stated that

high OTR films reduce the impact of unpleasant odours opening the package. Assay 1, due to its results should, therefore, be further scrutinized and the type of film used in meat packaging should be object of a more extensive study, as it is an important factor of influence in turkey meat products oxidation.

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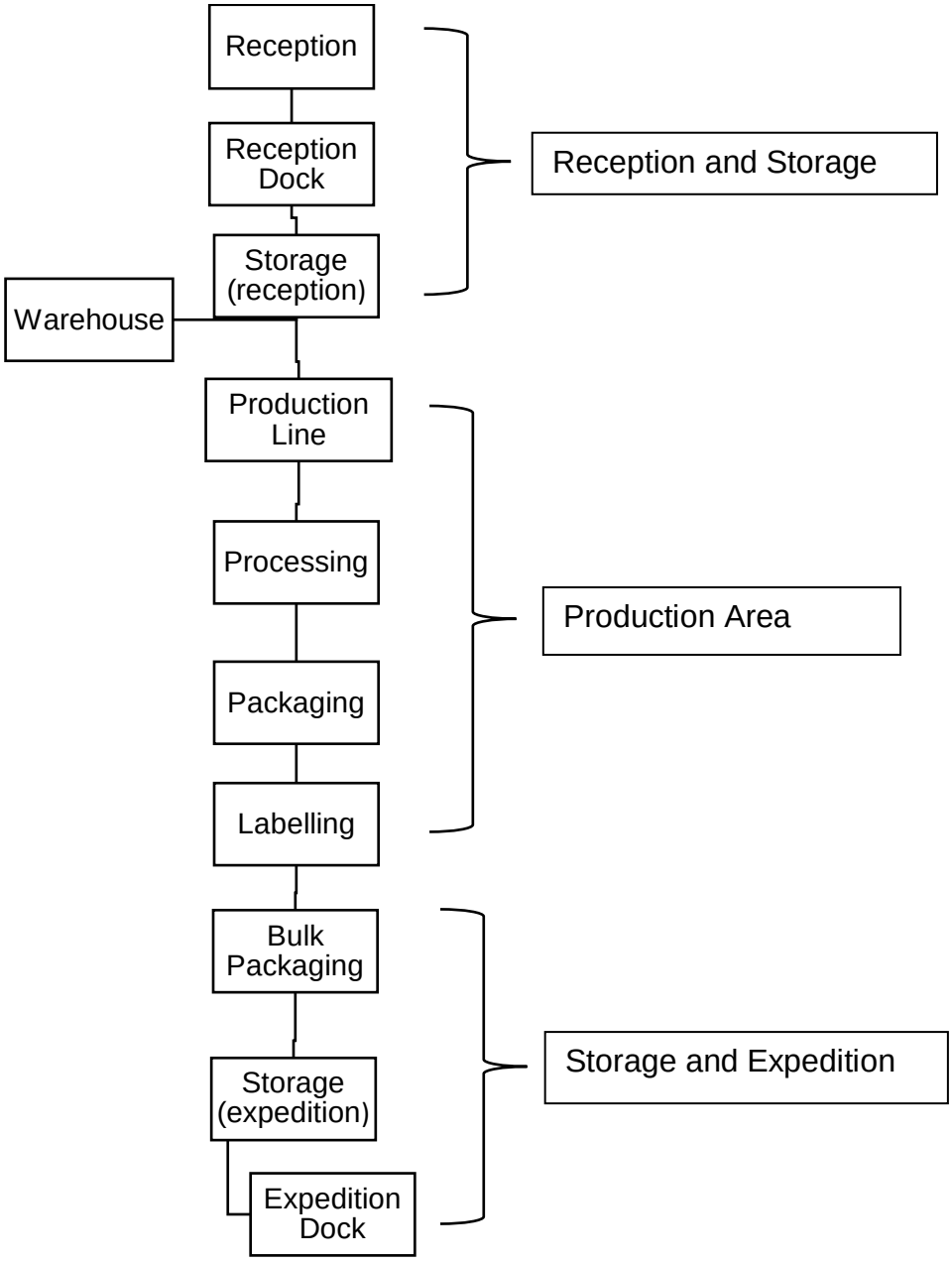
6. Annex/Appendix

I. Table with nutritional data for turkey thigh, from whole bird, meat only, raw (USDA, 2017).

Nutrient	Unit	Value per 100g (edible portion)
Water	g	76,0
Energy	kcal	108
protein	g	21,3
Total lipid (fat)	g	2,5
Carbohydrate	g	0,2
Fiber, total dietary	g	0,0
Sugars, total	g	0,1

II. Flowchart of the process with common steps to all turkey fresh meat products.



III. Processing flowchart of turkey skewers production.